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Identification and Targeting of Candidate Pre-Existing Lurker Cells that Give Rise to
Castration-Resistant Prostate Cancer

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14. ABSTRACT Castration-resistant prostate cancer (CRPC) is a lethal disease. We lack standard biomarkers to predict hormonal sensitivity of tumors, and we need new targets for therapy to prevent or treat CRPC. The selection theory predicts that pre-existing androgen-independent prostate cancer cells (termed lurker cells) contribute to CRPC, as they are not targeted by androgen deprivation therapy and may be capable of regenerating the tumor. Therefore, targeting these predicted lurker cells in combination with androgen-deprivation therapy would likely prevent or delay the onset of CRPC. The goals of this proposal are to provide functional evidence for intermediate luminal progenitor cells as the pre-existing "lurker" cells in primary prostate tumors, to evaluate potential therapeutic targets in intermediate luminal progenitor cells, and to define candidate biomarkers in intermediate luminal progenitor cells that can predict prognosis and response to hormonal therapy.					
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1. INTRODUCTION

Castration-resistant prostate cancer (CRPC) is a lethal disease. We lack standard biomarkers to predict hormonal sensitivity of tumors, and we need new targets for therapy to prevent or treat CRPC. The selection theory predicts that pre-existing androgen-independent prostate cancer cells (termed lurker cells) contribute to CRPC, as they are not targeted by androgen-deprivation therapy and may be capable of regenerating the tumor. Therefore, targeting these predicted lurker cells in combination with androgen-deprivation therapy would likely prevent or delay the onset of CRPC. The goals of this proposal are to provide functional evidence for intermediate luminal progenitor cells as the pre-existing “lurker” cells in primary prostate tumors, to evaluate potential therapeutic targets in intermediate luminal progenitor cells, and to define candidate biomarkers in intermediate luminal progenitor cells that can predict prognosis and response to hormonal therapy.

2. KEYWORDS

Prostate, epithelial, Androgen-deprivation therapy, Castration-resistant prostate cancer, luminal progenitor, intermediate cell, lurker cell

3. ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1. Interrogation of human I/LP cells as candidate lurker cells. Time frame: months 1-24. *This Aim is complete using a new approach to evaluate castration-resistance of selected populations.*

Task 1. Test the in vivo effects of castration on human luminal cancer subsets isolated from primary regenerated prostate tumors.

Task 2. Test the in vivo growth capacity of human luminal cancer subsets isolated from primary clinical prostate tumors.

Task 3. Test the in vivo effects of castration on human luminal cancer subsets isolated from primary clinical prostate tumors.

Specific Aim 2. Proof of principle therapeutic targeting of I/LP cells to prevent CRPC. Time frame: months 1-36. *This aim is 75% complete with new data for Tasks 4 and 5.*

Task 4. Determine if I/LP cancer cells from primary clinical human prostate tumors express genes associated with CRPC.

Task 5. Determine regulators of I/LP clonogenic activity in vitro.

Task 6. Target I/LP cells in vivo.

Specific Aim 3. Diagnostic potential of I/LP cells as lurker cells to predict CRPC development.

Time frame: months 1-36. *This aim is 60% complete as Task 7 is accomplished while we have new data on Tasks 8 and 9.*

Task 7. Determine whether I/LP cell transcriptional signature can predict patient prognosis.

Task 8. Testing of candidate I/LP biomarkers on prostate cancer tissue.

Task 9. Determine if I/LP biomarkers can predict hormone sensitivity.

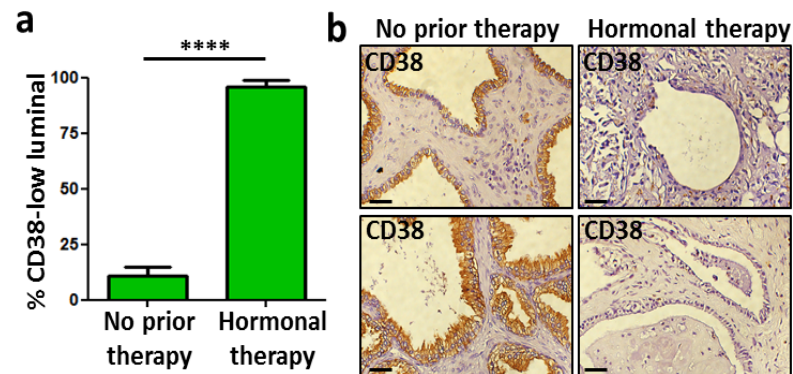
What was accomplished under these goals?

Specific Aim 1. Interrogation of human I/LP cells as candidate lurker cells.

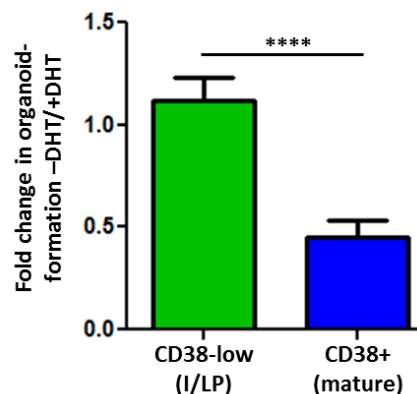
1) major activities: We were able to evaluate clinical tissues from patients undergoing hormonal therapy compared to control patients with no prior therapy and analyze the frequency of cells with a mature luminal or intermediate/progenitor phenotype, allowing us to measure the castration-resistance of each subset in humans. We also measured castration-resistance of isolated subsets in vitro in the presence or absence of DHT in a clonogenicity assay.

2) specific objectives: (1) Test the in vivo effects of castration on human luminal cancer subsets isolated from primary regenerated prostate tumors, (2) Test the in vivo growth capacity of human luminal cancer subsets isolated from primary clinical prostate tumors, (3) Test the in vivo effects of castration on human luminal cancer subsets isolated from primary clinical prostate tumors.

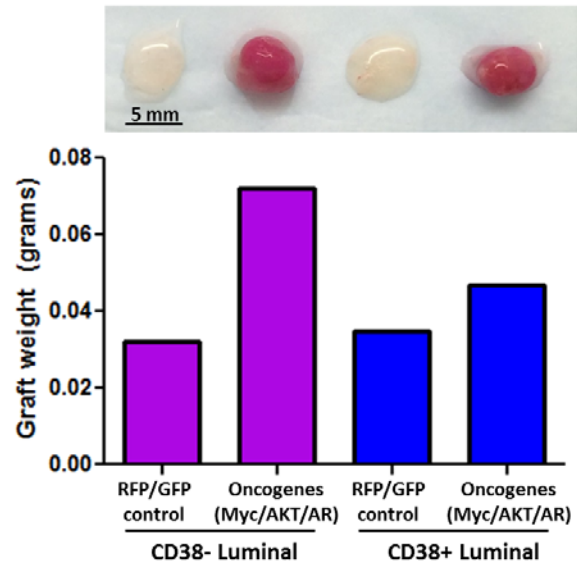
3) significant results or key outcomes: We determined that there is a dramatic enrichment for the intermediate/luminal progenitor phenotype indicating that this subset is expanded after castration and demonstrates castration-resistance (Figure 1 below: (a) Percentage of luminal cells with CD38-low Intermediate/progenitor phenotype in benign glands from 7 patients undergoing hormonal therapy compared to 30 patients without prior therapy. Two-tailed unpaired T-test with **** $p < 0.0001$. (b) Immunohistochemical staining for CD38 in representative glands from patient specimens corresponding to plot in (a). Scale bars, 50 microns).



In vitro growth in the absence of DHT also demonstrates castration-resistant properties of the intermediate/luminal progenitor cells compared to the mature luminal phenotype (Figure 2 below: fold change in organoid formation for intermediate/progenitor CD38-low and mature CD38+ luminal subsets without DHT compared to DHT-containing media, demonstrating castration-resistance).



4) other achievements: We found that tumors initiated by CD38-low intermediate/progenitor tumors were larger than tumors initiated by CD38+ mature cells suggesting that I/LP cells are stronger drivers of tumor initiation. (Figure 3 below: CD38-low and CD38+ luminal subsets were transduced with fluorescent control lentivirus RFP/GFP or oncogene-expressing lentivirus Myc, AKT, AR and transplanted into mice. The resulting tumors are shown on top with tumor weight shown below. CD38-low luminal tumors were dramatically larger and heavier than CD38+ tumors, indicating a more aggressive phenotype from this subset.)

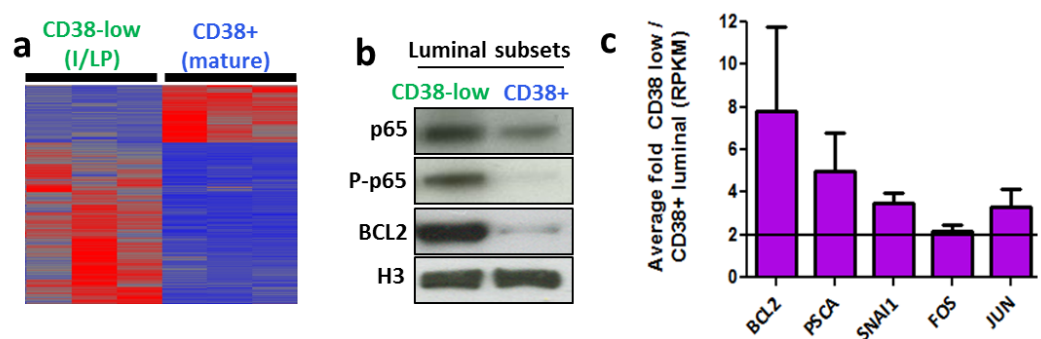


Specific Aim 2. Proof of principle therapeutic targeting of I/LP cells to prevent CRPC.

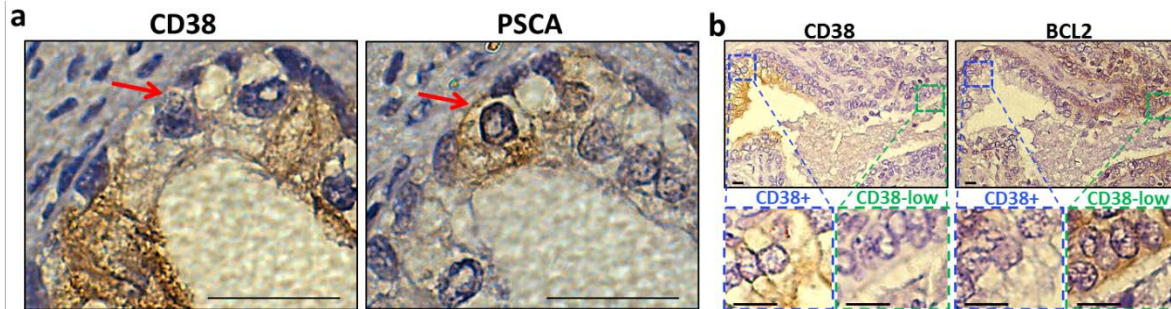
1) major activities: I/LP and remaining luminal cells from primary patient tissue have been analyzed at the RNA and protein levels. We have identified several players involved in aggressive prostate cancer or CRPC and confirmed their expression in I/LP-like cells.

2) specific objectives: (1) Determine if I/LP cancer cells from primary clinical human prostate tumors express genes associated with CRPC. (2) Determine regulators of I/LP clonogenic activity in vitro. (3) Target I/LP cells in vivo.

3) significant results or key outcomes: We have demonstrated that several genes/proteins implicated in disease progression and CRPC are highly expressed in I/LP cells including BCL2, PSCA, SNAI1, JUN, and FOS. We have confirmed expression of BCL2 and PSCA in I/LP-like cells at the protein level. Further analysis will be necessary to determine if targeting BCL2 or PSCA, each of which is in clinical trials as a therapeutic target, can be effective to eliminate these cells. (Figure 4 below: (a) Heat map of RNA sequencing results of CD38-low and CD38+ luminal subsets from three independent patients. (b) Western blot analysis of CD38-low and CD38+ luminal subsets from a representative benign human prostate sample stained for total p65, phosphorylated p65 (P-p65), BCL2 and Histone H3 as a loading control. (c) RNAseq analysis of genes associated with aggressive prostate cancer upregulated in CD38-low progenitor-like luminal cells. Average fold change of RPKM values in CD38-low vs CD38+ luminal cells from three independent patients. Line set at 2-fold enrichment in CD38-low progenitor-like luminal cells.)



4) other achievements: We have confirmed expression of BCL2 and PSCA in I/LP-like cells at the protein level. Further analysis will be necessary to determine if targeting BCL2 or PSCA, each of which is in clinical trials as a therapeutic target, can be effective to eliminate these cells. (See Figure 5 below: (a) Immunohistochemical analysis of benign human prostate stained for CD38 and prostate stem cell antigen (PSCA). Arrows point to CD38-low PSCA+ cells. Scale bars, 25 microns. (b) Immunohistochemical analysis of benign human prostate stained for CD38 and BCL2. Insets identify regions of high CD38/low BCL2 (left) and low CD38/high BCL2 (right, red arrows). Scale bars, 25 microns.)

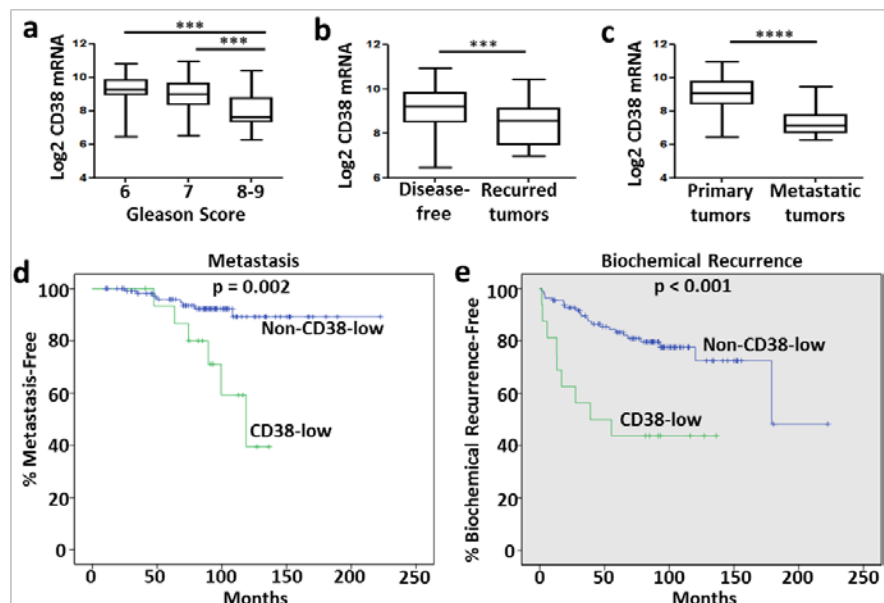


Specific Aim 3. Diagnostic potential of I/LP cells as lurker cells to predict CRPC development.

1) major activities: We have previously demonstrated that the gene signature of I/LP and non-progenitor luminal cells can stratify patient outcome in a Watchful Waiting cohort, demonstrating that the I/LP transcriptional signature has predictive power. We have now demonstrated the potential of several biomarkers to predict metastasis and biochemical recurrence

2) specific objectives: (1) Determine whether I/LP cell transcriptional signature can predict patient prognosis. (2) Testing of candidate I/LP biomarkers on prostate cancer tissue. (3) Determine if I/LP biomarkers can predict hormone sensitivity.

3) significant results or key outcomes: Using the cBio Cancer Genomics Portal, we found that low CD38 mRNA is associated with increasing Gleason score, metastasis and biochemical recurrence in the Memorial Sloan Kettering dataset (See Figure 6 below: (a-b) Correlation plots for CD38 mRNA (log2 whole transcript values) and Gleason Score (a) or Disease-free vs. Recurrence tumor status (b) in primary tumors containing mRNA. (c) Correlation of CD38 mRNA in primary vs. metastatic tumors using all tumors containing mRNA. Statistics represent Newman-Keuls multiple comparison test with *** $p < 0.0005$ (a) and Two-tailed unpaired T-test (b, c) with *** $p < 0.001$ and **** $p < 0.0001$. (d-e) Survival analysis measuring metastasis and biochemical recurrence for tumors containing CD38 mRNA 1 standard deviation below the mean compared to the remainder.)



Low CD38 mRNA is predictive of metastasis and biochemical recurrence in a statistically significantly manner regardless of whether CD38 is treated as a continuous variable or a categorical value. Importantly, low CD38 status is statistically significantly associated with both metastasis and disease recurrence after correction for nomogram score which includes both clinical and pathological variables. (See Table 1 below).

Univariate Cox Regression, **Biochemical Recurrence**, CD38 as a categorical variable

	Sig.	Exp (B)	95% CI for Exp (B)	
			Lower	Upper
CD38_zLessThanNeg1	0.001	3.665	1.685	7.972

Multivariate Cox Regression with nomogram, **Biochemical Recurrence**, CD38 as a categorical variable

	Sig.	Exp (B)	95% CI for Exp (B)	
			Lower	Upper
CD38_zLessThanNeg1	0.006	3.023	1.381	6.619
Biostats_nomogram_Stephanson	0.000	0.955	0.942	0.968

Univariate Cox Regression, **Metastasis**, CD38 as a categorical variable

	Sig.	Exp (B)	95% CI for Exp (B)	
			Lower	Upper
CD38_zLessThanNeg1	0.004	4.729	1.640	13.635

Multivariate Cox Regression with nomogram, **Metastasis**, CD38 as a categorical variable

	Sig.	Exp (B)	95% CI for Exp (B)	
			Lower	Upper
CD38_zLessThanNeg1	0.030	3.280	1.125	9.559
Biostats_nomogram_Stephanson	0.000	0.960	0.943	0.977

Univariate Cox Regression, **Biochemical Recurrence**, CD38 z-score as a continuous variable

	Sig.	Exp (B)	95% CI for Exp (B)	
			Lower	Upper
CD38_zLessThanNeg1	0.001	0.517	0.352	0.759

Multivariate Cox Regression with nomogram, **Biochemical Recurrence**, CD38 z-score as a continuous variable

	Sig.	Exp (B)	95% CI for Exp (B)	
			Lower	Upper
CD38_zLessThanNeg1	0.000	0.952	0.939	0.965
Biostats_nomogram_Stephanson	0.000	0.488	0.327	0.728

Univariate Cox Regression, **Metastasis**, CD38 z-score as a continuous variable

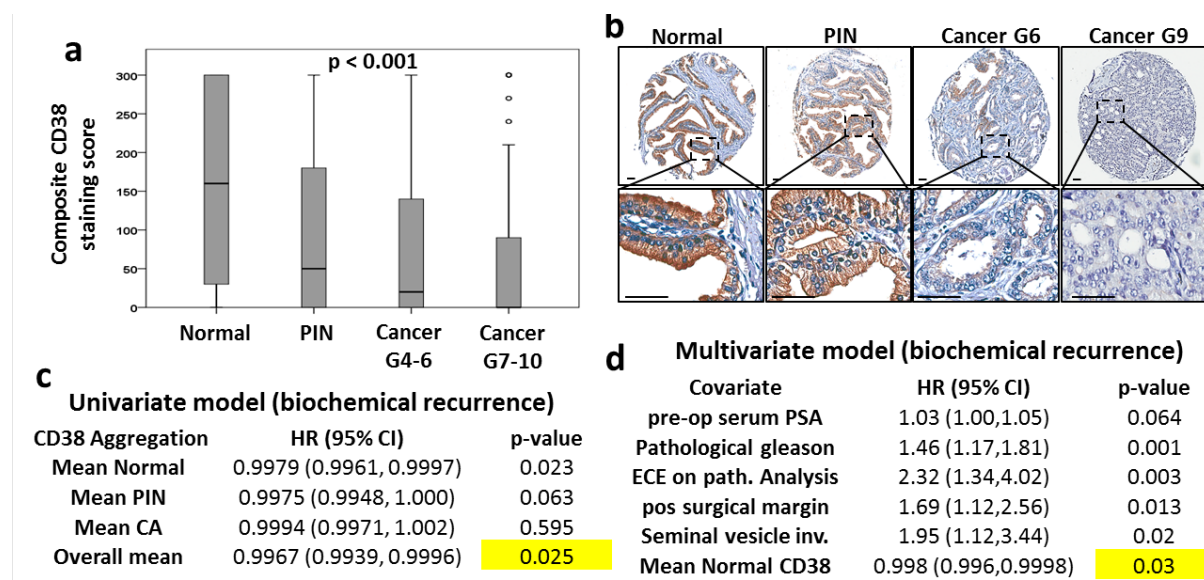
	Sig.	Exp (B)	95% CI for Exp (B)	
			Lower	Upper
CD38_zLessThanNeg1	0.002	0.394	0.220	0.708

Multivariate Cox Regression with nomogram, **Metastasis**, CD38 z-score as a continuous variable

	Sig.	Exp (B)	95% CI for Exp (B)	
			Lower	Upper
CD38_zLessThanNeg1	0.006	0.412	0.218	0.778
Biostats_nomogram_Stephanson	0.000	0.959	0.942	0.977

4) other achievements: In a tissue microarray containing tissue cores from 267 patients with prostate cancer, we found the highest protein expression of CD38 in benign glands, with reduced expression in PIN and prostate cancer. The lowest expression of CD38 was found in the most advanced tumors based

on Gleason grade. Immunohistochemical staining was verified using two different anti-CD38 antibodies. The average CD38 expression level was statistically significantly associated with time to biochemical recurrence even after controlling for known predictors of recurrence including PSA and Gleason score. (See Figure 7 below: (a) Plot of composite CD38 staining score in tissues histologically classified as normal, Prostate intraepithelial neoplasia (PIN), or prostate cancer with Gleason scores of 4-6, or 7-10. Middle bar represents mean value. The overall effect of different levels of CD38 among the prostate regions was confirmed ($p < 0.001$). (b) Representative immunohistochemical images of normal, PIN, Gleason 6 and Gleason 9 cancer stained for CD38 with high power images below. Scale bars, 50 microns. (c) Cox proportional-hazards model was used to demonstrate a statistically significant association between low expression of CD38 and time to biochemical recurrence ($p = 0.025$). (d) Multivariate model accounting for known predictors of biochemical recurrence demonstrates a statistically significant association of low CD38 expression in normal tissue with time to biochemical recurrence ($p = 0.017$). P-values < 0.05 were considered statistically significant.)



What opportunities for training and professional development has the project provided?

The Principal Investigator has taken part in group activities related to UCLA's SPORC in Prostate Cancer. The PI has also become a group leader for the Prostate Cancer Foundation Young Investigator Tumorigenesis Working Group, leading monthly conference calls involving young investigators from around the globe.

How were the results disseminated to communities of interest?

Results from this project have been presented to the UCLA campus through a Jonsson Comprehensive Cancer Center seminar and to the Prostate Cancer Foundation Young Investigator community through the Tumorigenesis Working Group monthly conference call presentation.

What do you plan to do during the next reporting period to accomplish the goals?

We plan to focus on biomarkers and therapeutic targets expressed by the intermediate/progenitor cells and test candidate biomarkers on tissue microarrays containing biochemical recurrence or hormone responsiveness outcome data.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Our findings offer a new potential biomarker or series of biomarkers to predict aggressive prostate cancer including metastasis and biochemical recurrence. We hope that these findings may be used to better understand the factors driving aggressive disease and be useful in the clinic.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS

Publications, conference papers, and presentations

Work describing the role of luminal progenitor-like cells has been presented in a number of seminars during the past year, at UCLA, Stanford, UC Irvine, UT Southwestern and online via the Prostate Cancer Foundation Young Investigator Tumorigenesis Working Group.

Goldstein AS. (2014) An origin story: epithelial progenitor cells, inflammatory signaling and prostate cancer. Canary Center at Stanford for Cancer Early Detection, Palo Alto, CA.

Goldstein AS. (2014) Prostate cancer as a disease of progenitor cells. Children's Medical Center Research Institute at UT Southwestern, Dallas, TX.

Goldstein AS. (2015) Prostate cancer as a disease of progenitor cells. UC Irvine Department of Biological Chemistry, Irvine, CA.

Goldstein AS. (2015) Prostate cancer as a disease of progenitor cells. UCLA SPORE/Jonsson Comprehensive Cancer Center GU Oncology Leaders Lecture, Los Angeles, CA.

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers, and presentations.

Nothing to Report

Website(s) or other Internet site(s)

Nothing to Report

Technologies or techniques

Nothing to Report

Inventions, patent applications, and/or licenses

Nothing to Report

Other Products

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name: Andrew Goldstein

Project Role: PI

Nearest Person Month Worked: 3.2

Contribution to Project: Tumor development in Aim 1, biomarker investigation in Aim 3

Name: Xian Liu

Project Role: Technician/Staff Research Associate

Nearest Person Month Worked: 6.6

Contribution to Project: Tumors and castration studies in Aim 1, study and identification of BCL2 and PSCA as potential mechanisms promoting castration-resistance, biomarker investigation in Aim 3

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Steve Horvath contributed to statistical analysis in year 1 but was not needed in Year 2 or 3 as was indicated on the initial budget and therefore did not contribute person months in Year 2.

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

N/A

9. APPENDICES

N/A